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TEST PLAN FOR SULFOSUCCINATES CATEGORY (Revised)

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May 13, 2002OVERVIEW

The Sulfosuccinates Group (SSG) of the Synthetic Organic Chemical Manufacturers Association. (SOCMA) hereby submits for review a test plan for a category consisting of three sulfosuccinates under the Environmental Protection Agency's (EPA) High Production Volume (HPV) Chemical Challenge Program. It is the intent of the panel and its member companies to use existing data on one or more of the sulfosuccinates to adequately fulfill the Screening Information Set (SIDS) for environmental fate endpoints, ecotoxicity tests, and human health effects for all three sulfosuccinates. The Sulfosuccinates Group believes that adequate data exist to fulfill all the requirements of the HPV program without the need for additional testing.

Test Plan Matrix for Sulfosuccinates

Chemical	Cyclohexyl (CAS # 23386-52-9)	Dimethylbutyl (CAS # 2373-38-8)	Ethylhexyl (CAS # 577-11-7)
PHYSICAL CHEMISTRY			
Melting point	Y	E	Y
Boiling point	NA	NA	NA
Vapor Pressure	NA	NA	NA
Water Solubility	Y	Y	Y
Kow	E	E	E
ENVIRONMENTAL FATE			
Photodegradation	E	E	E
Stability in Water	E	E	E
Biodegradation	Y	Y	Y
Transport between Environmental Compartments (Fugacity)	E	E	E
ECOTOXICITY			
Acute Toxicity to Fish	Y	Y	Y
Acute Toxicity to Aquatic Invertebrates	Y	C	Y
Toxicity to Aquatic Plants	Y	C	C
TOXICOLOGICAL DATA			
Acute Toxicity	Y	Y	Y
Repeated Dose Toxicity	Y	Y	Y
Genetic Toxicity-Mutation	Y	C	Y
Genetic Toxicity- Chromosomal Aberrations	C	C	Y
Carcinogenicity	C	C	Y
Toxicity to Reproduction	Y	Y	Y
Developmental Toxicity	C	C	Y
OTHER TOXICITY DATA			
Human Experience	NR	NR	Y
Pharmacokinetics	NR	NR	Y

Y = adequate experimental data ; NA = not applicable;

E = Endpoint fulfilled via EPIWIN model.

C = endpoint fulfilled by category approach; NR = not required

TABLE OF CONTENTS

1.	Information about the Panel.....	3
2.	Category Analysis	3
2.1	Identity of Category Members	3
2.2	Background Information on Category Members	3
2.3	Chemical Reactivity and Metabolism.....	5
3.	Test Plan.....	5
3.1	Chemical and Physical Properties.....	6
3.1.1	Melting Point	6
3.1.2	Boiling Point	6
3.1.3	Vapor Pressure	7
3.1.4	Octanol/Water Partition Coefficients.....	7
3.1.5	Water Solubility.....	7
3.1.6	Test Plan for Physical Properties	7
3.2	Environmental Fate and Pathways	7
3.2.1	Photodegradation	8
3.2.2	Stability in Water	8
3.2.3	Biodegradation.....	8
3.2.4	Fugacity.....	9
3.2.5	Test Plan for Environmental Fate Parameters	9
3.3	Ecotoxicity.....	10
3.3.1	Acute Toxicity to Fish	10
3.3.2	Acute Toxicity to Aquatic Invertebrates	10
3.3.3	Acute Toxicity to Aquatic Plants.....	11
3.3.4	Acute Toxicity to Terrestrial Plants.....	11
3.3.5	Other.....	11
3.3.6	Test Plan for Ecotoxicity.....	12
3.4	Human Health Data.....	12
3.4.1	Acute Toxicity.....	12
3.4.2	Repeated Dose Toxicity.....	12
3.4.3	Genetic Toxicity.....	14
3.4.4	Carcinogenicity	14
3.4.5	Reproductive Toxicity.....	14
3.4.6	Developmental Toxicity.....	15
3.4.7	Human Experience	16
3.4.8	Test Plan for Mammalian Toxicity	16
3.5	Conclusion	16
4.	References	18
5.	Appendix 1 - Criteria for listing of robust summaries	23
6.	Appendix 2 - Robust Summaries	23

1. Information about the Panel

The Sulfosuccinates Group is formed under the sponsorship of the Synthetic Organic Chemical Manufacturers Association (SOCMA). The Panel consists of the following manufacturers of sulfosuccinates:

Crompton Corporation	MFG Chemical, Inc.
Cytec Industries Inc.	Rhodia Inc.
Finetex Inc.	Uniqema
McIntyre Group, Ltd.	

2. Category Analysis

2.1 Identity of Category Members

The substances included in the Sulfosuccinate Category are as follows:

Succinic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt Designated as "Ethylhexyl ester."	CAS No. 577-11-7
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Succinic acid, sulfo-, 1,4-bis(1,3-dimethylbutyl)ester, sodium salt Designated as "Dimethylbutyl ester."	CAS No. 2373-38-8
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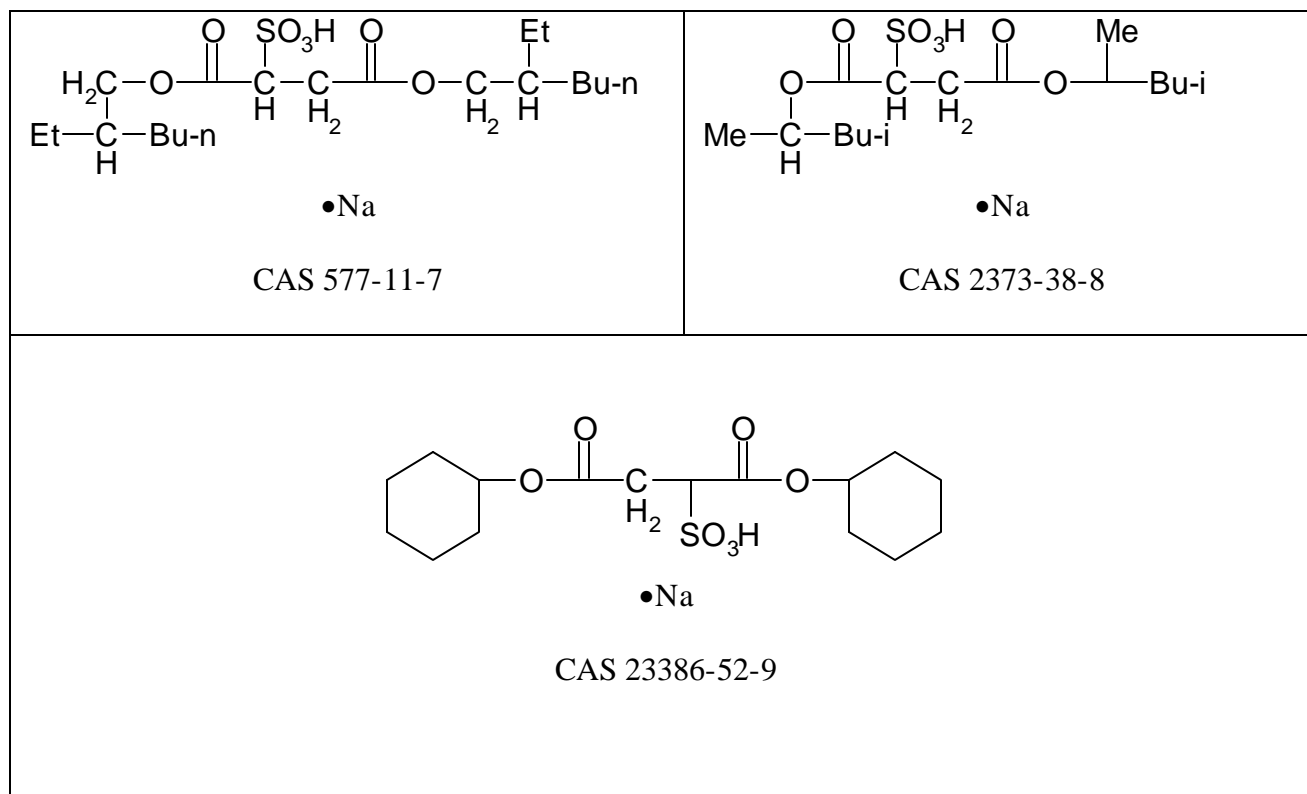
Succinic acid, sulfo-, 1,4-bis(dicyclohexyl)ester, sodium salt Designated as "Cyclohexyl ester."	CAS No. 23386-52-9
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2.2 Background Information on Category Members

The Sulfosuccinates Category consists of three sulfosuccinate esters as designated above. The molecular structures of all three category members are closely related. The general structure for the category is defined as "dialkyl sodium sulfosuccinate" or "dicycloalkyl sodium sulfosuccinate." This describes a molecule with a succinic ester backbone, in which a carbon alpha to one of the carboxyl functions has a sodiumsulfo group in place of a hydrogen atom. The only structural difference in the three substances is the alcohol moiety of the ester function. The different alcohol groups are 2-ethylhexyl-, cyclohexyl- and 1,3-dimethylbutyl. The generic molecular structure of all category members is shown below:

$\text{ROOCCH}_2\text{CH}(\text{SO}_3\text{Na})\text{COOR}$, Where $\text{R} = \text{2-ethylhexyl- } [\text{CH}_3(\text{CH}_2)_3\text{CH}(\text{CH}_2\text{CH}_3)\text{CH}_2\text{-}]$
 $= \text{1,3-dimethylbutyl- } [(\text{CH}_3)_2\text{CHCH}_2\text{CH}(\text{CH}_3)\text{-}]$
 $= \text{cyclohexyl- } [\text{cyclic } -(\text{CH}_2)_5\text{CH-}]$

The structures are as follows:



The three substances are grouped together because of their close structural relationships and the resulting similarities of their physiochemical and toxicological properties. They are marketed as solids or solutions in various alcohols.

The ethylhexyl ester is also known as dioctyl sodium sulfosuccinate or docusate sodium. It is generally regarded as safe when used as a stool softener and when used to lower surface tension and produce a mucolytic effect. The usual dosage for these indications is 50 to 250 mg daily for adults and children over 12, and 50 to 150 mg for children aged 2-12 (AMA, 1983). As of March 1994, dioctyl sodium sulfosuccinate was reported to be used in 44 cosmetic formulations (FDA, 1994). Concentrations of use are no longer reported to the FDA (Federal Register, 1992). However, FDA data from 1984 report dioctyl sodium sulfosuccinate concentrations in a variety of cosmetics at $\leq 5\%$ (FDA, 1984). Dioctyl sodium sulfosuccinate can be used up to 15 ppm in finished gelatin desserts, 10 ppm in finished beverages or fruit juice drinks, 25 ppm in molasses, 25 ppm in non-carbonated beverages containing cocoa fat, 0.5% by weight in gums and hydrophilic colloids, and 9 ppm in finished products when used as a diluent in color additive mixtures for food (CFR, 2000; CIR, 1996). Dioctyl sodium sulfosuccinate can also be used as an adjuvant in tablets and in vitamin preparations, as well as for non-FDA uses described below for the other category members.

The cyclohexyl- and dimethylbutyl esters are used as surfactants or wetting agents, and as ingredients in some adhesives, polymeric coatings, and detergents. The cyclohexyl ester is cleared for use in food-contact applications under CFR Section 178.3400 ("Emulsifiers and/or surface-active agents") as sodium 1,4 dicyclohexyl sulfosuccinate, without any limitations. Because neither of these agents is used as direct food additives or in stool softeners, their potential for oral exposure is expected to be less than that of the ethylhexyl ester.

2.3 Chemical Reactivity and Metabolism

The category members are all chemically stable at room temperature and neutral conditions. They are not particularly sensitive to oxidation, except in the presence of strong oxidizers. They are stable for long periods in aqueous systems, but are expected to undergo saponification (cleavage of the ester groups) in the presence of strong base.

Metabolic studies in animals indicate that the ethylhexyl ester is absorbed and metabolized to some extent after oral administration. Within 24-48 hours of oral administration, 25-35% of ³⁵S-labeled, and 64.1% of ¹⁴C-labeled ethylhexyl ester are excreted into urine of rats (Patel et al., 1969; Kelly, 1973). Up to 89% of an orally administered dose is excreted into urine of rabbits (Kelly, 1973). The metabolic profile in the rabbit suggests that it is absorbed intact rather than being hydrolyzed in the GI tract prior to absorption. In dogs, 25.5 % and 71.1% of ¹⁴C-labeled ethylhexyl ester is excreted into urine and feces, respectively, suggesting a lower degree of absorption in the dog than the rat (Kelly, 1973). In humans given 100 mg or 200 mg orally, dioctyl sodium sulfosuccinate is present in bile at concentrations of 2-4 x 10⁻⁵ M (Dujovne and Shoeman, 1972).

From 15.5 to 18.6 % of an orally administered dose (5 to 10 mg) of ¹⁴C-labeled ethylhexyl ester to rats is excreted into urine as 2-ethylhexanol-forming compounds. Other metabolites found in the urine of rats given the ethylhexyl ester include maleic and fumaric acid (Kelly et al., 1973), which can be formed by the oxidation of succinate by succinic dehydrogenase (Stryer, 1981). Compounds found in the urine of dogs include unmetabolized ethylhexyl ester and incompletely hydrolyzed ester derivatives. In humans, excretion of 2-ethylhexanol into urine accounts for 2.5-5.0 % of an administered dose (200 mg) (Kelly et al., 1973).

Based on the data obtained for the ethylhexyl ester and the structural similarities between this chemical and the cyclohexyl and dimethylbutyl esters, it is likely that the cyclohexyl and dimethylbutyl esters are also absorbed to some extent after oral administration. It is also likely that these esters will be metabolized in rodents by esterases. Compounds formed from de-esterification will be similar for all three molecules, with the exception of the alcohol moiety. Whereas de-esterification of sodium diethylhexyl sulfosuccinate gives rise to 2-ethylhexanol, similar metabolism of sodium dicyclohexyl sulfosuccinate leads to the formation of cyclohexanol. Likewise, metabolism of sodium 1,3-dimethylbutyl sulfosuccinate leads to methyl isobutyl carbinol. It should be noted that 2-ethylhexanol has already been reviewed in the OECD/SIDS program and designated as low priority for further work. Cyclohexanol and methyl isobutyl carbinol are also being sponsored individually in SIDS or other HPV Chemical

programs. These programs serve as the appropriate vehicles for characterizing the toxicology of these metabolites.

3. Test Plan

3.1 Chemical and Physical Properties

All three category members can be considered organosulfo salts. As neat materials, therefore, they are solids with high melting points, negligible volatility (vapor pressure). When heated above 300° C, they will undergo decomposition instead of boiling. All members are slightly to very slightly soluble in water due to the presence of the sodium sulfo group, which enhances hydrophilicity. However, due to the presence of two 6- and 8-carbon alkyl groups in the ester function, water solubility is limited, and affinity to lipids and hydrophobic materials is enhanced. For this reason, solubility in aqueous media is enhanced by the added presence of water-miscible solvents such as low molecular weight alcohols. Chemical/physical properties are summarized in Table 1.

Table 1. Chemical/physical properties of sulfosuccinates

Endpoint	Cyclohexyl ester (CAS # 23386-52-9)	Dimethylbutyl ester (CAS # 2373-38-8) ¹	Ethylhexyl ester (CAS # 577-11-7)
Melting point (° C)	203 ² 311.3 ³	349.84 ³	153-7 ² 162.5 - 168.5 ³
Boiling point ⁴	N/A	N/A	N/A
Vapor pressure	Negligible (salt)	Negligible (salt)	Negligible (salt)
Partition coefficient (Log Pow or Kow)	1.76 ³	1.8371 ³	3.95 ³
Water solubility (g/l at 25 ° C)	120 ²	300–320 ²	15 ²

¹Values shown above are for neat substances. The dimethylbutyl ester is marketed as marketed as a solution in water/alcohol. This material has a volatility and boiling point consistent with water/alcohol.

²Measured

³Estimated by EPIWIN

⁴Will decompose before boiling on heating to high temperatures

3.1.1 Melting Point

The EPIWIN model (Table 1) predicts that all category members have high melting points. That is consistent for organic salts in general. Measured melting points have been determined for the 2-ethylhexyl and cyclohexyl esters.

3.1.2 Boiling Point

The boiling points of all category members in the form of the neat product are not applicable because these materials are salts, and will degrade when heated to above 300°C. The boiling

point of a marketed form of the dimethylbutyl ester containing 69-73% sodium dimethylbutyl ester, 4% ethanol, 2% methyl isobutyl carbinol and water is 173° F (78° C), which corresponds to the boiling point of the alcohol present.

3.1.3 Vapor Pressure

The vapor pressures of all three category members are negligible, consistent with them being organic salts. The dimethylbutyl ester marketed as a solution of water/alcohol has a vapor pressure consistent with that of water/alcohol.

3.1.4 Octanol/Water Partition Coefficients

The log Pow (Kow) values for the three succinate esters have been estimated using the EPIWIN program algorithms. These are 1.76 for the cyclohexyl ester, 1.8371 for the dimethylbutyl ester, and 3.95 for the ethylhexyl ester. The differences in log Kows correlate roughly with the length of the alkyl chains in the ester function.

3.1.5 Water Solubility

With the neat salt of the three sulfosuccinates, the physical form is as a waxy solid, as would be expected for an organic salt with alkyl sidechains. With water present, the physical form will be part solid, part liquid, and will go into solution if a water-miscible organic solvent is present. The solubility of ethylhexyl ester in water is 15 g/l at 25° C, 23 g/l at 40° C, 30 g/l at 50° C, and 55 g/l at 70° C (Windholz, 1983). Water solubility values supplied by the manufacturer for the cyclohexyl ester and dimethylbutyl ester at 25° C are 120 g/l and 300-320 g/l, respectively (Cytec Industries Inc., 2001).

3.1.6 Test Plan for Physical Properties

Pertinent physical property values have been determined either through measurement or estimations using models, such as EPIWIN. As organic salts, the category members will have moderate to high melting points, will decompose at elevated temperatures and not boil, and will exhibit negligible vapor pressures. The measured solubility values for category members indicate high solubility, and are consistent for expectations for sodiosulfo organic salts. The commercial products for all three category members are commonly sold as aqueous mixtures, sometimes with ethanol or isopropyl alcohol added to promote complete solubilization. In these cases, the physical properties will correspond to the mixtures and reflect the presence of water or alcohol present. No additional physical property determinations are needed.

3.2 Environmental Fate and Pathways

Results of environmental fate studies with the three sulfosuccinates are summarized in Table 2.

Table 2. Environmental fate studies with sulfosuccinates

Endpoint	Cyclohexyl ester, (CAS # 23386-52-9)	Dimethylbutyl ester, (CAS # 2373-38-8)	Ethylhexyl ester, (CAS # 577-11-7)
Photolysis (Atmospheric $T_{1/2}$)	5.2 hours	7.3 hours	5.6 hours
Photolysis (Hydroxyl Radical Rate Constant)	24.6 E-12 $\text{cm}^3/\text{molecule-sec}$	17.4 E-12 $\text{cm}^3/\text{molecule-sec}$	22.9 E-12 $\text{cm}^3/\text{molecule-sec}$
Stability in Water	1.45 years @ pH8; 14.5 years @ pH7	15.6 years @ pH8; 156 years @ pH7	243 days @ pH 8; 6.7 yr @ pH7
Biodegradation ¹	35.9% after 28 days (Shake flask)	40.3% after 28 days (Shake flask); 16.7% after 28 days (Closed bottle)	66.7% after 28 days (Closed bottle)
Koc	111	57.6	1040
Henry's Law Constant	3.14E-13 atm-m ³ /mole (EPIWIN)	1.62E-12 atm-m ³ /mole (EPIWIN)	5.02E-12 atm-m ³ /mole (EPIWIN)

All values were derived from the EPIWIN model (except biodegradation)

¹Biodegradation data are for a marketed form of dimethylbutyl ester containing 80% CAS # 2373-38-8, 15% water and 5% ethanol.

3.2.1 Photodegradation

The results of EPIWIN modeling (Table 2) indicate that all three sulfosuccinates are degraded by photolysis to a similar extent. Atmospheric photodegradation is not expected to be an important elimination pathway, since the category members, as organic salts, will not volatilize significantly.

3.2.2 Stability in Water

The EPIWIN model predicts that these succinate salts are stable to hydrolysis in water with half-lives estimated at several years (Table 2). The dimethylbutyl ester is estimated to hydrolyze more slowly in water than the other sulfosuccinates. Since all three category members are esters, hydrolysis to sodio-sulfo succinic acid and the corresponding alcohols could occur under strongly acid or basic conditions, especially at elevated temperatures. The commercial products have long been commonly sold as aqueous solutions, providing practical evidence to the stability of category members in water under neutral, ambient conditions.

3.2.3 Biodegradation

Results of experiments OECD guideline studies will all three sulfosuccinates also indicate moderate rates of biodegradation. Results of shake flask tests indicate 35.9% biodegradation of the cyclohexyl ester and 40.3% biodegradation of a marketed form of the dimethylbutyl ester

after 28 days (United States Testing Company, Inc. 1988a,b). The closed bottle (United States Testing Company, Inc., 1991a) test indicates a lower rate of biodegradation of a marketed form of the dimethylbutyl ester (16.7%) than the shake flask test (40.3%). The ethylhexyl ester had a higher rate of biodegradation than the other two sulfosuccinates (66.7% by 28 days in the closed bottle test)(United States Testing Company, Inc., 1991b).

A study by Vrbanova et al. (1999) suggests that the initial rates of biodegradation of sulfosuccinate esters increases with increasing length of the alkyl chain up to the C-8 ester, and that the substitution of cyclohexyl for n-hexyl results in a 4-fold decrease in the rate of biodegradation (Vrbanova et al., 1999). Further analyses revealed that the primary factors influencing the rate of biodegradation of linear sulfosuccinates are the number of carbons on the chain (rather than branching) and the degree of hydrophobicity (surfactants with medium hydrophobicity decompose more rapidly than the highly hydrophobic or hydrophilic ones). Based on this analysis, the cyclohexyl and dimethylbutyl esters should degrade more slowly than the ethylhexyl ester. Results of the OECD studies confirm this relationship.

3.2.4 Fugacity

The Mackay Level III fugacity model allows the estimation of relative distributions of chemicals released into the environment, but does not predict actual environmental concentrations. Distributive models, such as the MacKay Level III model, assume zero loss of material through degradation or dispersion out of the environmental system. The MacKay Level III model predicts that all three succinate salts will partition primarily to soil/sediment, some to water and a negligible portion to air (Table 3).

Table 3. MacKay Level III fugacity model

Medium	Cyclohexyl ester (CAS # 23386-52-9)	Dimethylbutyl ester (CAS # 2373-38-8)	Ethylhexyl ester (CAS # 577-11-7)
	Concentration %	Concentration %	Concentration %
Air	0.875	0.911	1.55
Water	40.18	38.7	37.3
Soil	58.2	68.3	59.9
Sediment	0.1	0.101	1.33

Level III fugacity modeling predicts that soil is the preferred environmental compartment for category members, followed next by water. Differences between category members are relatively small. The model probably overestimates air concentrations, since organic salts have negligible volatility.

3.2.5 Test Plan for Environmental Fate Parameters

All endpoints have been met by experimentation or use of EPIWIN. No further testing is required.

3.3 Ecotoxicity

Results of ecotoxicity studies with the three sulfosuccinates are summarized in Table 4.

Table 4. Ecotoxicity Studies with Sulfosuccinates

Endpoint	Cyclohexyl ester, (CAS # 23386-52-9)	Dimethylbutyl ester, (CAS # 2373-38-8)	Ethylhexyl ester, (CAS # 577-11-7)
Acute toxicity to fish	96 hr LC ₅₀ (bluegill) = 470 mg/l	96 hr LC ₅₀ (bluegill, trout) > 1000 mg/l; 1200 mg/l	96 hr LC ₅₀ (bluegill, trout) = 37 mg/l; 28 mg/l
Acute toxicity to Daphnia	48 hr EC ₅₀ = 457 mg/l	ND	48 hr EC ₅₀ = 36.2 mg/l
Toxicity to algae	No EC ₅₀ determined – Growth stimulated at concentrations < 1000 mg/l	ND	ND
Phytotoxicity	NOEL (24, 48 hr) =10 mmol/l; 1.25 mmol/l	ND	NOEL (24, 48 hr) = 0.625 mmol/l; < 0.3125 mmol/l
Bioconcentration Factor (BCF) ¹	3.162	3.162	1.750

ND – not determined experimentally. Fish toxicity data for the dimethylbutyl ester are for a marketed form containing 80% CAS # 2373-38-8, 15% water and 5% ethanol.

¹ values were obtained by EPIWIN

3.3.1 Acute Toxicity to Fish

Acute toxicity studies in fish have been performed for all three sulfosuccinates. The LC₅₀ values for the ethylhexyl ester in two different species of fish range from 28- 37 mg/l (Analytical Biochemistry Laboratories, 1987a, Goodrich et al., 1991; Goodrich/Huber/Lech, 1985; United States Testing Company, 1990a). The LC₅₀ value for the cyclohexyl ester is approximately one order of magnitude higher (470 mg/l)(Analytical Biochemistry Laboratories, 1987b), and the LC₅₀ value for a marketed form of the dimethylbutyl ester in two different species is approximately 1000 g/l (Analytical Biochemistry Laboratories, 1987c; United States Testing Company, Inc. 1990b). The range of LC₅₀ values for the sulfosuccinates correlates roughly with the length of side chain.

3.3.2 Acute Toxicity to Aquatic Invertebrates

Data are available for two of the sulfosuccinates (ethylhexyl and cyclohexyl)(Goodrich/Lech, 1985; Exxon Biomedical Sciences, Inc. 1993a). The 48-hour EC₅₀ values for effects on *Daphnia* for the ethylhexyl ester (36.2 mg/l) and the cyclohexyl ester (457 mg/l) do not differ significantly from their corresponding 96 hr-LC₅₀ values determined for fish. Therefore, it is expected that the 48-hour EC₅₀ value for exposure of *Daphnia* to the dimethylbutyl ester would be similar to its 96 hr-LC₅₀ value for fish (approximately 1000 mg/l).

3.3.3 Acute Toxicity to Aquatic Plants

Algal toxicity data are available for the cyclohexyl ester. Incubation of *Selenastrum capricornutum* with 8.1, 90 and 300 mg/l cyclohexyl ester for 96 hours stimulates the growth rate by 64.5, 57.8 and 38.2%, respectively, and growth by 164, 243, and 86.4%, respectively (Exxon Biomedical Sciences, Inc, 1993b). The growth rate and growth of algae incubated with 1000 mg/l cyclohexyl ester was similar to control. The fact that this surfactant stimulates algal growth at lower concentrations is not surprising, since some surfactants also have been shown to stimulate growth at concentrations lower than those that are toxic (Lewis, 1990). This action is thought to be due to the surfactant increasing permeability of the membrane to nutrients. The reported concentrations that are stimulatory and toxic vary depending on the type of surfactant. The fairly toxic coconut ether ethoxylate causes stimulation and inhibition of growth at 0.003 mg/l and 0.050 mg/l, respectively; whereas the fairly nontoxic linear alkylbenzene sulfonate causes stimulation and inhibition of growth at 500 mg/l and > 500 mg/l, respectively (Lewis, 1990). Based on results of the tests with these surfactants, it is not unreasonable to assume that concentrations greater than 1000 mg/l cyclohexyl ester would produce toxicity to algae, and that the other members of the category would stimulate algal growth at lower concentrations and inhibit growth at higher concentrations.

Based on results of test with fish, the dimethylbutyl ester would not be expected to be more toxic to algae than the cyclohexyl ester. Therefore, it is reasonable to assume that this surfactant would also stimulate algal growth at concentrations < 1000 mg/l. Since the ethylhexyl ester is approximately 10-fold more toxic in fish, daphnia and terrestrial plants (see below) than the cyclohexyl ester, it is reasonable to assume that it will be approximately 10-fold more potent than the cyclohexyl ester in causing stimulation, then inhibition of growth of algae. Based on this assumption, the concentrations of ethylhexyl ester likely to cause stimulation, and then inhibition of algal growth are up to 100 mg/l, and > 100 mg/l, respectively. We believe that such an estimation is reasonable, and obviates the need for testing.

3.3.4 Acute Toxicity to Terrestrial Plants

Data are available for two of the sulfosuccinates (ethylhexyl and cyclohexyl). The toxicity of these sulfosuccinates to *Tradescantia bicolor* (Wandering Jew) follows the same type of relationship as was observed with fish and *Daphnia* – the ethylhexyl ester is more toxic (NOEL (24 hr) = 0.625 mmol/l) than the cyclohexyl ester (NOEL (24 hr) = 0.626 mmol/l) (Oros et al. 1999). Analyses that Oros and coworkers made with several sulfosuccinic acid esters showed that by decreasing the lipophilicity of the molecules, cyclization and branching of the alkyl chain decreased the toxicity.

3.3.5 Other

The bioconcentration factors (BCF) of the three sulfosuccinates are estimated to range from 1.75 to 3.16, indicating a low potential to bioconcentrate.

3.3.6 Test Plan for Ecotoxicity

No new ecotoxicity testing is recommended. Fish toxicity studies have been performed with all three sulfosuccinates and *Daphnia* toxicity studies have been performed on the cyclohexyl- and ethylhexyl sulfosuccinates. Toxicity to terrestrial plants is an important endpoint for the sulfosuccinates, since they can partition to soil. The pattern of toxicity to terrestrial plants is similar to that of fish and *Daphnia* (i.e. the ethylhexyl member is approximately 10 times more potent than the others).

Based on the structural similarities of the molecules and the weight of the evidence, the EC50 value for toxicity of the dimethylbutyl ester to *Daphnia* should not be less than that of the cyclohexyl ester (470 mg/l). The results of the algal toxicity test with the cyclohexyl ester should be predictive of the dimethylbutyl ester and the ethylhexyl ester (albeit at different concentrations). Based on the results of the fish, *Daphnia* and plant studies, the ethylhexyl ester would be expected to be approximately 10-fold more potent than the cyclohexyl ester in inducing, and then inhibiting algal growth.

3.4 Human Health Data

3.4.1 Acute Toxicity

Oral LD₅₀ values have been reported for all three chemicals in the category (dimethylbutyl as marketed form)(Table 5). In rats, the oral LD₅₀ values range from 1.75 - 4.2 g/kg, indicating a low degree of oral acute toxicity (American Cyanamid, 1957, 1966, 1969; Olson et al., 1962; Huntingdon Research Center, 1977). Values obtained in mice (2.6 - 4.3 g/kg) (Hopper et al., 1949; Case et al., 1977) and rats (2 - 4.2 g/kg) for the ethylhexyl ester are similar. There is no significant difference between the LD₅₀ values for all three compounds, indicating a similar degree of acute oral toxicity.

Dermal LD₅₀ values also have been reported for all three chemicals in the category. The values range from 5 ml/kg (4 g/kg) for a marketed form of the dimethylbutyl ester, to > 10 g/kg for the ethylhexyl ester, indicating a low degree of dermal acute toxicity (American Cyanamid, 1957, 1969; Huntingdon Research Center, 1977; Vernon et al. 1990).

3.4.2 Repeated Dose Toxicity

Oral repeated dose toxicity studies have been performed on all three sulfosuccinates. Results of 32-day studies in rats indicate a NOEL of $\geq 1.0\%$ for the cyclohexyl ester and $\geq 0.5\%$ for the marketed form of the dimethylbutyl ester (American Cyanamid, 1957, 1969). The results of 90-day studies in rats indicate NOELs of $\geq 1\%$ dietary for all three sulfosuccinates (Industrial Bio-Test Laboratories, 1969). Longer term oral toxicity studies in rats (16 or 26 weeks) have shown

Table 5. Mammalian toxicity of sulfosuccinates

Endpoint	Cyclohexyl ester, (CAS # 23386-52-9)	Dimethylbutyl ester, (CAS # 2373-38-8)	Ethylhexyl ester, (CAS # 577-11-7) ¹
Acute oral	LD ₅₀ (rat) = 3.54 g/kg ²	LD ₅₀ (rat) = 1.75 g/kg ²	LD ₅₀ (rat) = 2 g/kg; 3.08 g/kg; 4.2 g/kg LD ₅₀ (mouse) = 2.643 g/kg; 4.8 g/kg
Acute dermal	LD ₅₀ (rabbit) > 5 g/kg ²	LD ₅₀ (rabbit) = 5 ml/kg ² (4 g/kg as contained solids)	LD ₅₀ (rabbit) > 10 g/kg
Repeated dose (32 day)	NOEL(rat) > 1.0% ²	NOEC(rat) > 0.5 % ²	ND
(90 day)	NOEL(oral rat) > 1.0% dietary	NOEL(oral rat) > 1.0% dietary	NOEL (oral rat) > 1.0% dietary
(16 weeks)	ND	ND	NOEL (oral feed) < 2% dietary
(26 weeks)	ND	ND	NOEL (oral rat) = 0.5% dietary; LOEL (oral rat) = 1.0% dietary
(1 year)	ND	ND	NOEL (oral beagle) = 30 mg/kg
Genetic toxicity (in vitro)	Ames test - negative	ND	Ames test – negative CHO cells – positive only at cytotoxic conc.
Carcinogenicity	ND	ND	NOEL (oral rat) = 0.5% dietary; LOEL (oral rat) = 1.0% dietary; reduced weight gain
Reproductive toxicity	NOEL (oral rat) > 1.0% dietary for reproductive organs	NOEL (oral rat) > 1.0% dietary for reproductive organs	NOEL (oral rat) = > 1% dietary for reproductive organs; 1.0% dietary for reproductive effects; < 0.5% dietary for lactation
Developmental toxicity	ND	ND	NOEL (oral rat) = 1.0% dietary; LOEL (oral rat) = 2.0% dietary

ND = not determined

¹Also referred to as dioctyl sodium sulfosuccinate. Data are reported from studies that used “dioctyl sodium sulfosuccinate”, but not “n-dioctyl sodium sulfosuccinate”²A marketed form of the material containing 80% CAS # and 6-8% ethanol was used in the study

NOELs of < 2% and 0.5%, respectively (Fitzhugh 1948; Taylor 1966). The only effects noted in rats treated with 2% for up to 26 weeks were GI irritation and reduced weight gain. Daily oral

administration of 30 mg/kg ethylhexyl ester for 1 year produces no adverse effects in dogs (Case et al., 1977). Taken together, these results suggest that all three sulfosuccinates are fairly well tolerated when administered repeatedly.

3.4.3 Genetic Toxicity

The cyclohexyl ester has been tested for mutagenicity in Salmonella strains TA-98, TA-100, TA-1530, TA-1535, TA 1538 and WP-2uvrA- in the absence of S9 (American Cyanamid, 1976), and the ethylhexyl ester has been tested in strains TA-98, TA-100, TA-102, TA-1535, TA-1537 and TA-1538 in the absence and presence of S9 (Bonin and Baker, 1980; Hazelton Microtest, 1993a). The ethylhexyl ester was tested at the highest concentrations that did not produce cytotoxicity. Results of both studies were negative. A chromosomal aberration assay in Chinese Hamster Ovary cells (CHO) has been conducted with the ethylhexyl ester (Hazelton Microtest, 1993b). In one out of three experiments, 120 micrograms/ml ethylhexyl ester induced significant chromosomal aberrations (24/100 cells scored) in the presence of S-9 activation. The majority were abnormalities other than chromosomal gaps. Toxicity at the concentration that produced aberrations (120 µg/ml) was demonstrated as a 62% reduction in mitotic activity. Complete toxicity at doses exceeding 140 µg/ml was observed. In summary, the ethylhexyl ester only produced aberrations in 1/3 experiments at a concentration close to the toxic threshold. This is considered to be a negative result by Loveday et al. (1990).

Metabolites formed from de-esterification of the category members such as 2-ethylhexanol (from sodium diethylhexyl sulfosuccinate), cyclohexanol and methyl isobutyl carbinol have also been tested for genetic toxicity. 2-ethylhexanol and cyclohexanol are not mutagenic or cytogenic (Kirby et al., 1983; Phillips et al., 1982; Putman et al., 1983; Haworth et al., 1983; Industrial Health Foundation, 2001). Methyl isobutyl carbinol is not mutagenic in the Ames test (BIBRA, 1994). Chromosome aberration studies for methyl isobutyl carbinol were not located. Methyl isobutyl carbinol will be reviewed under the HPV Chemical program, and (as previously noted) cyclohexanol is also undergoing similar review. Therefore, the most appropriate approach for toxicological characterization of these metabolites is in their own separate HPV Chemical review processes.

3.4.4 Carcinogenicity

Long-term studies (up to 2 years) in rats with the ethylhexyl ester have shown that a dietary concentration of 1% produces no adverse effects except reduced weight gain (Fitzhugh and Nelson, 1948). Gastrointestinal irritation is noted in rats ingesting 2% ethylhexyl ester in the diet for 2 years, and ingestion of 8% produces severe GI irritation and lethality within a week (Fitzhugh and Nelson, 1948).

3.4.5 Reproductive Toxicity

Two three-generation reproductive toxicity experiments of have been performed on the

ethylhexyl ester (American Cyanamid, 1970; Hazleton Laboratories, 1986; Mackenzie et al., 1990). In each of the experiments, a dietary level of 0.5% was shown to affect parental food consumption, parental and fetal body weight of most generations. However, doses of up to 1.0% had no effect on fertility and gestation. Ingestion of 2.0% ethylhexyl ester in the diet on days 6-16 of gestation is associated with growth retardation in dams and a significant increase in fetal resorptions (Hoechst Roussel, 1976, 1979). In the reproductive toxicity study by American Cyanamid (1970), ingestion of 1% was associated with decreased lactation index of F0 and F2 dams and survivability of the F3 generation. In this study, test diet of some of the dams was replaced with regular diet just prior delivery and during lactation, and their offspring were placed on test diets after weaning. With the exception of the F1b pups, no effects of up to 1.0% ethylhexyl ester on viability, mean weight, or lactation were noted in pups from dams that did not receive DSS during lactation. This suggests that either the ability of dams to produce milk or the taste of the milk was affected by ingestion of ethylhexyl sulfosuccinate during lactation. Evidence to support this hypothesis comes from the finding in the Hazleton study (wherein all dams were given test diet during lactation) of dose-dependent increases in the number of pups with no milk in their stomachs.

Results of 90-day studies show that ingestion of up to 1.0% of any of the sulfosuccinates in the category has no effect on reproductive organs of male or female rats (Industrial Bio-Test Laboratories, 1969). These data support a lack of reproductive toxicity for the members of the category.

3.4.6 Developmental Toxicity

In the three generation reproductive toxicity studies mentioned above, no developmental toxicity was observed in pups born of rats treated with ethylhexyl ester at concentrations up to 1.0% (American Cyanamid, 1970; Hazleton Laboratories, 1986; Mackenzie et al., 1990). No adverse effects are noted in offspring of rats given 1.0% ethylhexyl ester in the diet on days 6-15 of gestation (Hoechst Roussel, 1976). Ingestion of 2.0% ethylhexyl ester in the diet on days 6-15 of gestation is associated with an increased percentage of malformed fetuses (20% versus 0% in controls (Hoechst Roussel, 1976). Abnormalities in fetuses include exencephaly, spina bifida, microphthalmia, curved or open vertebral columns, and incomplete ossification of various cranial bones. An additional study performed at 2.0% also indicates that this dose is associated with an increase in skeletal abnormalities (Hoechst Roussel, 1979; Mattison, 1984). The effects noted at this concentration are associated with maternal toxicity as evidenced by growth retardation and a significant increase in fetal resorptions. Based on the available data and the structural similarities of the compounds, it can be surmised that the cyclohexyl and dimethylbutyl esters would also produce maternal and subsequent developmental toxicity at 2.0%.

2-Ethylhexanol, a metabolite of the 2-ethylhexyl ester in rodents, has been extensively studied and reviewed in the OECD/SIDS program. 2-Ethylhexanol has been shown to exhibit developmental toxicity in rodents only at high oral doses that are maternally toxic (USEPA, 1990; Hardin et al., 1987; Nelson et al., 1990; Tyl et al., 1992; NTP, 1991). The alcohol metabolite of sodium bis 1,3-dimethylbutyl sulfosuccinate, methyl isobutyl carbinol, is slated for

review as a high production volume chemical. Cyclohexanol is undergoing a similar review. The data being presented in these reviews are available for those interested in further information regarding the toxicity endpoints (including developmental effects) of these metabolites. The general knowledge that aliphatic esters are metabolized to the alcohols in rodents lends further support to the category justification for mammalian endpoints, and the demonstrated lack of significant developmental toxicity of these metabolites, including 2-ethylhexanol, as well as the low developmental toxicity of sodium diethylhexyl sulfosuccinate itself, suggest that neither the parent molecules nor their expected metabolites are developmental toxicants.

3.4.7 Human Experience

A retrospective study on drug use of 6,837 women during pregnancy indicates that use of dioctyl sodium sulfosuccinate during pregnancy is not associated with an increased risk of birth defects in offspring (Jick et al., 1981).

3.4.8 Test Plan for Mammalian Toxicity

Results of acute and repeated dose toxicity studies indicate that the members of the category are well tolerated and are not reproductive toxicants. They do not appear to have differential toxicity in mammals, as the LD50 and NOAEL values in rats are similar. Developmental toxicity studies of the 2-ethylhexyl ester and a possible metabolite (2-ethylhexanol) indicate that these materials are not toxic at doses that are not maternally toxic. Ames tests with two of the category members (ethylhexyl and cyclohexyl) and a chromosomal aberration test with the ethylhexyl ester indicate that these materials are not genotoxic. The toxicology of metabolites arising from de-esterification of the cyclohexyl- and dimethylbutyl- esters (i.e. cyclohexanol and methyl isobutyl carbinol) is currently under review. It is more appropriate to refer to the separate HPV Chemical review processes for these chemicals than to conduct a separate assessment for these metabolites in this test plan. No additional testing is necessary.

3.5 Conclusion

Physical Properties

As stated in Section 2.2, the three chemical substances that comprise the Sulfosuccinates Category all have a common molecular structure. Each category member has a molecular structure that consists of a succinic ester backbone, in which a carbon alpha to one of the carboxyl functions has a sodium sulfo group in place of a hydrogen atom. The only structural difference in the three substances is the alcohol moiety of the ester functions. The different alcohol groups are 2-ethylhexyl-, cyclohexyl- and 1,3-dimethylbutyl-.

All three category members have similar physical properties. As neat materials they are all solid salts with high melting points, and negligible vapor pressure. Because they are salts, they will degrade when heated to high temperatures (>300° C) and not boil.

Environmental Fate

The category members are predicted to undergo photolysis in the atmosphere, with half lives estimated to range from 5.2-7.3 hours. Atmospheric photodegradation is not believed to be an important route of elimination, since all three category members are organic salts with negligible volatility. All members are predicted to be stable to hydrolysis in neutral water, but will undergo cleavage of the ester group in the presence of strong base. Biodegradation studies indicated that the succinate esters biodegrade at moderate rates. The Log K_{ow}s are estimated at 1.76 for the cyclohexyl ester, 1.84 for the dimethyl butyl ester, and 3.95 for the ethylhexyl ester, which correlate roughly with increasing chain length of the alkyl ester group. Water solubility tends to decrease with increasing side chain length, while K_{oc} values (which predict soil mobility) tend to increase with chain length. Thus, the 2-ethylhexyl ester appears to be the least water soluble, to have the greatest lipophilicity, and (with the highest K_{oc} value) appears to have the least mobility in soil. The predicted Henry's Law constants for the three sulfosuccinates are low (<1 E-11 atm-m³/mole). That is consistent with the negligible vapor pressure and significant solubility of salts.

The MacKay Level III fugacity model predicts a similar relative environmental distribution for all three category members, indicating negligible distribution to air, moderate distribution to water, and high distribution to soil and sediment.

Ecotoxicity

The ethylhexyl ester is more toxic to aquatic species than the cyclohexyl ester. Based on studies which indicate that the ecotoxicity of the sulfosuccinates is governed by the length of the side chain, the dimethylbutyl ester is expected to behave more like the cyclohexyl ester than the ethylhexyl ester. The bioconcentration factor (BCF) of the three sulfosuccinates are estimated to range from 1.75 to 3.16, indicating a low potential to bioconcentrate.

Mammalian Toxicity

Results of 90-day repeated dose oral toxicity experiments indicate NOELs of > 1.0% for all three sulfosuccinates. Results of these studies indicate that none of the materials are toxic to reproductive organs. Ingestion of 1% ethyl hexyl ester during lactation reduces the lactation index (either by reducing the ability of dams to produce milk or adversely affecting the taste of the milk). A maternally-toxic dose of 2.0% ethylhexyl ester causes developmental toxicity. Based on the structural similarities of the molecules, tests performed on the ethylhexyl ester should be predictive of results for the other sulfosuccinates. The toxicology of the alcohol metabolites (cyclohexanol and methyl isobutyl carbinol) is currently under HPV Chemical review in their own programs.

Summary

In summary, the data provided in the robust summaries and test plan are consistent with the close molecular similarity of the category members. No new testing is required.

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5. Appendix 1 - Criteria for listing of robust summaries

Robust summaries for all HPV endpoints were written from all available data with the following exceptions:

Ethylhexyl ester (CAS #577-11-7) - A biodegradation study by Hammerton (1955) was not summarized because its conduct would not meet today's standards. Toxicity studies performed by Benaglia et al (1943) on rats, rabbits, monkeys and dogs and were not summarized because the results were not well documented, the number of animals was not sufficient, or the NOEL was difficult to determine. Results of a study by Hopper et al. (1949) in mice ($LD_{50} = 4.8 \text{ g/kg}$) also were not summarized because the conduct of the study would not be acceptable by today's standards. Physical chemistry and fish toxicity data (48 hr LC_{50} in killifish of 61.3 mg/l) from CITI also were not included because the primary source of information was unknown. All studies described in these references would be assigned a reliability of 3 (based on the standards of Klimisch et al., 1997).

6. Appendix 2 - Robust Summaries